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Thermal inactivation of *Listeria monocytogenes* in ground beef under isothermal and dynamic temperature conditions

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ABSTRACT

The objective of this research was to compare the suitability of three kinetic models for describing the survival of a cocktail of *Listeria monocytogenes* in ground beef under both isothermal and dynamic temperature conditions. Ground beef (93% lean), inoculated with a 4-strain cocktail of *L. monocytogenes*, was subjected to heating at 57, 60, 63, or 66 °C to develop isothermal kinetic models. Experimental data showed that the isothermal survival curves were not strictly linear and were downwardly concaved. The isothermal inactivation of *L. monocytogenes* in ground beef was better described by two nonlinear kinetic models, the Weibull-type and the modified Gompertz models. Analytical results showed that root mean square error values (RMSE) of the Weibull-type and the modified Gompertz models were 0.19 and 0.20 log(CFU/g), both significantly smaller than that of the linear model (0.48 log(CFU/g)). Under linear heating dynamic conditions, however, only the modified Gompertz model, with a RMSE of only 0.71 log(CFU/g), was suitable for describing the survival of the pathogen. Both linear and Weibull-type models grossly underestimated the survival of *L. monocytogenes* in ground beef during dynamic heating.
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1. Introduction

Thermal processing involves using high temperature to inactivate spoilage and pathogenic microorganisms in foods, and is one of the most effective and widely used technologies for food preservation. With sufficient time and temperature used in thermal processing, products with commercial sterility or an extended shelf-life can be made available in large quantities for distribution and consumption. The major drawback of thermal processing is that it may cause products to lose color, flavor, texture, or nutritional values while the spoilage microorganisms and foodborne pathogens are destroyed in the process. Over-processing has been a major complaint of this technology, which in part may be attributed to inaccurate kinetic models under certain circumstances (Huang and Juneja, 2001).

The establishment of a thermal process for food preservation and the evaluation of its effectiveness are based upon the kinetics of bacterial inactivation concerning the target microorganism for a known time–temperature history. Numerous experimental evidences have suggested that the destruction of microorganisms (spores or vegetative cells) in foods generally follows a linear manner, i.e., the log counts of bacteria would decrease linearly with time under an isothermal condition. Historically, the first-order kinetics has been used to describe such a process (Stumbo, 1973). Under a constant temperature, the process of thermal inactivation can be mathematically expressed as

$$\frac{dC}{dt} = -kC. \tag{1}$$

In Eq. (1), the rate of change (dC/dt) in the number or concentration (C) of the target microorganism in a food substrate decreases linearly with C under an isothermal condition. The coefficient k is a temperature-dependent rate constant. This equation can be integrated to produce a general equation for an isothermal thermal inactivation process:

$$\ln(C) = \ln(C_0) - kt. \tag{2}$$

In food science, however, the concentration term in Eq. (2) has been more conveniently expressed as the logarithm of base 10, and Eq. (2) further becomes

$$\log(C) = \log(C_0) - \frac{t}{D}. \tag{3}$$

Plotting the logarithm (base 10) of the survivor counts against the heating time should yield a semi-logarithmic linear curve. Apparently D is equal to $\ln(10)/k$, or $2.303/k$, which is the time needed to inactivate 90% of the cells in a population. D, a constant under an isothermal condition, is a function of temperature and is conventionally expressed in a log-linear relationship with respect to heating temperature (T), a reference temperature (T_{ref}), and the D-value at the reference temperature (D_{ref}):

$$\log(D) = \log(D_{ref}) - \frac{1}{z}(T - T_{ref}), \quad \text{or } D = D_{ref}10^{-\frac{T-T_{ref}}{z}}. \tag{4}$$

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For most microorganisms, the linear inactivation kinetics works quite well. However, even in the early days of research, nonlinear inactivation curves have been observed and documented (Stumbo, 1973; Whithell, 1942; Williams et al., 1941). A range of nonlinear survivor curves of different shapes has been reported in the literature (Moats, 1971; Casolari, 1988; Huang and Juneja, 2001; Juneja et al., 2006). According to Plug and Holocomb (1983) approximately 2/3 of the semi-logarithmic survivor curves of homogeneous cultures are not linear. In the data analyzed by van Boekel (2002), it was reported that the majority of the thermal inactivation curves (53 out of 55 cases) were not linear. Recently, a new approach, employing the concept of Weibull frequency distribution of the viability of spores and vegetative cells during thermal processing, has been proposed as an alternative to linear thermal inactivation kinetics (Peleg and Cole, 1998; Mafart et al., 2002; van Boekel, 2002). Regardless the meaning of the frequency distribution of viability of spores or vegetative cells of bacteria in a population, which is rather difficult to observe, examine, or validate experimentally, the physical form of the Weibull-type model can be derived directly from the standard linear model and expressed in a nonlinear equation (Huang and Juneja, 2001):

$$\log(C) = \log(C_0) - Kt^{\alpha}. \quad (5)$$

The model expressed in Eq. (5) is more general than the traditional linear kinetics (Eq. (3)), and allows the survivor curves to "bend" upward or downward, depending on the value of α. If $\alpha > 1$, which enhances the effect of time on bacterial kill, the curve expressed by the equation would bend downward and thus may be used to describe the shoulder effect or downwardly concaved curves. If $\alpha < 1$, the curve would bend upward, easing the effect of time on thermal inactivation in the model, and is more suitable to describe the "tail effect". If $\alpha = 1$, the equation is reduced to the normal first-order linear kinetics. Therefore this model can be more flexible and realistic, and more accurate for describing nonlinear survivor curves (Albert and Mafart, 2005; van Boekel, 2002; Huang and Juneja, 2001). Apparently, the coefficient K affects the rate of bacterial inactivation, and α determines the shape of an inactivation curve under isothermal conditions.

Mathematical approaches of using the more general Weibull-type model for estimating the lethality of non-isothermal dynamic processes also have been reported by several researchers (Chen et al., 2007; Corradini et al., 2005; Halder et al., 2007; Mafart et al., 2002; Peleg et al., 2001; Peleg and Penchina, 2000). However, in almost all these publications the survivor data used in the calculation were taken either from the published literature, or generated artificially by simulation. Although many hypothetic approaches and thermal curves have been suggested, few of these approaches have been directly validated using experimental data. The accuracy of these approaches and the applicability of the Weibull-type models for estimating dynamic processes of thermal inactivation have not been experimentally tested and validated.

In a recent experiment to determine the thermal inactivation kinetics of *Listeria monocytogenes* in ground beef, it was observed that the survivor curves were apparently not linear and exhibited downward concavity. The experimental observations presented a unique opportunity to directly compare the linear inactivation kinetics with the Weibull-type model. Therefore, the objective of this research was to compare the accuracy and applicability of the Weibull-type model with the traditional first-order kinetics under both isothermal and non-isothermal dynamic temperature conditions.

2. Materials and methods

2.1. Bacteria strains

Four strains of *L. monocytogenes* (H7763, H7776, H7778, and 46877), all isolates from actual foodborne illness outbreaks associated with meat products, were used in this study. The bacteria were propagated and properly maintained on Tryptic Soy agar (TSA, BD/Difco Laboratories, Sparks, MD) plates and stored at 4 °C in a refrigerator.

The bacteria cultures were prepared by individually inoculating each strain to 10 ml brain heart infusion broth (BHI broth, BD/Difco Laboratories, Sparks, MD), incubated at 37 °C on an orbital shaker (100 rpm), and harvested after approximately 24 h of incubation. The bacteria cultures were centrifuged in a refrigerated centrifuge (15 min at 2400*g*), washed with 10 ml 0.1% sterile peptone water (PW, BD/Difco Laboratories, Sparks, MD), re-centrifuged, re-suspended in 1 ml PW, and then combined to form a 4 ml cocktail. The bacterial concentration was approximately $10^{9.5}$ CFU/ml in the final cocktail. In recent years multiple-strains of bacterial cocktails have been preferred over single individual strains in determining the thermal inactivation kinetics and lethality of foodborne pathogens in raw and RTE meats (Murphy et al., 2004a; Juneja et al., 2003; Murphy et al., 2003a,b; Smith et al., 2001). Therefore, bacterial cocktail was used in this study.

2.2. Sample preparation and inoculation

Ground beef (93% lean), purchased from a local grocery store, was divided into ~35 g portions, vacuum-sealed in plastic bags (Polynylon, 0.08 mm in thickness), and stored in a freezer (~−20 °C). Before an experiment, one bag of sample was thawed overnight in a refrigerator (~4 °C). After thawing, the ground beef was combined with previously prepared bacteria cocktail and mixed twice in a stomacher mixer (Model BagMixer® 100 W, Interscience Co., France) at maximum speed for 15 min.

After inoculation, the samples were divided into 1.00 ± 0.05 g portions and individually placed into filter bags (Whirl-Pak®, 7 oz, 95 mm × 180 mm × 0.08 mm, NASCO – Fort Atkinson, Fort Atkinson, WI). Each ground beef sample in the filter bags was flattened with a round bottle to <1 mm in thickness and vacuum-sealed at 1200 Pa (12 mbar) to allow the removal of the internal air and to ensure uniform heat transfer. The samples were kept on ice prior to the thermal inactivation studies.

2.3. Thermal inactivation

The inoculated samples were subject to heating under isothermal conditions in a circulating water bath (Fisher Scientific Isotemp Model 1016S, Pittsburgh, PA) maintained at 57, 60, 63, or 66 °C. The come-up time for the samples in the water bath was previously determined as 6 s (data not shown), which was excluded from the total heating time. Samples were fully submerged under hot water for thermal inactivation, with heating time ranging from 10 to 1980 s, depending on the temperature. Except at 57 °C, duplicate samples were submerged under hot water for each time–temperature combination. This procedure was taken to allow unrestricted heating of the samples. For heating at 57 °C, samples were loosely distributed in the water bath and duplicated samples were periodically removed. Thermal inactivation was terminated by immediately placing the heat-treated samples into an iced water bath (~1.5 °C). Each time–temperature combination was repeated at least three times.

2.4. Determination of bacterial counts

Filter bags containing heat-treated or control samples were aseptically opened and added with 5 ml PW, placed in the stomacher mentioned previously, and stomached twice, each for 3 min at maximum speed. After that, the samples were plated, either directly or after serial dilution, onto freshly prepared PALCAM *Listeria* selective agar (BD/Difco Laboratories, Sparks, MD)

plates (van Netten et al., 1989). Prior to being transferred to a 37 °C incubator, the PALCAM plates were allowed to remain at room temperature for approximately 2 h, allowing the resuscitation of thermally injured cells. After approximately 48 h incubation, typical *Listeria* colonies were counted, averaged, and converted to the logarithm (base 10) of CFU per gram of ground beef. Although selective agar was used, two studies have shown that PALCAM allowed recovery and growth of thermally injured *Listeria* cells (Miller et al., 2006; Williams and Golden, 1998).

2.5. Kinetic analysis

2.5.1. Linear kinetics

For linear kinetics, linear regression was used to correlate the log counts of *L. monocytogenes* to heating time using Eq. (3). The linear regression was conducted using a statistical analysis package – NCSS-2000 (Hintze, 1998). The *D*-value under an isothermal condition was calculated from the inverse of the slope of the regression curve. The *z*-value of the bacteria cocktail was calculated from the inverse of the slope of the regression curve between the logarithm (base 10) of *D* and the heating temperature using Eq. (4).

2.5.2. Weibull-type model

Nonlinear regression was used to correlate the log counts of bacteria to time using Eq. (5) and to obtain the parameters K and α. A nonlinear regression procedure of NCSS-2000 was used to perform the nonlinear analysis. After nonlinear regression was completed, a pseudo-R^2 was calculated by NCSS-2000 using the following equation:

$$R^2 = 1 - \frac{\sum_1^n (Y_i - \widehat{Y}_i)^2}{\sum_1^n (Y_i - \overline{Y})^2}, \tag{6}$$

where Y_i is the $\log_{10}$ counts of bacteria, $\widehat{Y}_i$ is the $\log_{10}$ counts estimated by the model, $\overline{Y}$ is the average of the $\log_{10}$ counts of bacteria, and n is the number of data points of an inactivation curve.

2.5.3. Modified Gompertz model

The modified Gompertz model has been widely used to describe the isothermal growth kinetics of microorganisms in foods (Gibson et al., 1987; Huang, 2003a; Juneja et al., 1999). This model was adopted for describing the isothermal inactivation kinetics of *L. monocytogenes* in ground beef. Nonlinear regression (NCSS-2000) was used to fit the inactivation curves to the following equation:

$$\log(C) = \log(C_0)\{1 - \exp[-\exp(-\mu(t - M))]\}, \tag{7}$$

where μ is the relative inactivation rate (s^{-1}); and M is a time constant (s).

Although a freeware tool was available to assess non-log-linear microbial survivor curves (Geeraerd et al., 2005), the modified Gompertz model was not listed in the freeware. For the sake of consistency, both linear and nonlinear regression tools available in the statistical package NCSS-2000 was used to analyze the experimental data.

2.5.4. Comparison of models

To compare the accuracy of the models developed under isothermal conditions, a statistical parameter, the square root of the mean squared error (RMSE) was calculated for each kinetic model (Eq. (8)).

$$\mathrm{RMSE} = \sqrt{\frac{1}{n}\sum \left(Y_i - \widehat{Y}_i\right)^2} \tag{8}$$

Analysis of variance (ANOVA) was conducted to compare of the mean of RMSE among different models. The Tukey's studentized range (HSD) test procedure was used to group the means of RMSE (alpha = 0.05). The statistical analysis was conducted using SAS version 6.12 (SAS Institute, Cary, NC).

2.5.5. Validation of kinetic models under a dynamic condition

A dynamic heating experiment was designed to validate the isothermal inactivation kinetic models developed in this study. Ground beef samples were subjected to linear heating in a circulating water bath (Model ESRB-7, Techne Inc., Burlington, NJ). The water bath temperature was automatically controlled using a program developed by Huang (2003b) to provide linear heating of samples. The water bath temperature was programmed to increase linearly from 30 °C at 1.72 °C/min, which was the highest heating rate achievable in the experiments. Samples, separated approximately 10 mm apart, were loaded into the water bath and submerged under hot water. With the come-up-time (6 s) excluded from the total heating time, samples were retrieved from the hot water bath at predetermined sampling intervals. The heating was terminated by immediately placing the treated samples under iced water. The bacteria were recovered and plated using the same procedure described above. Any heating profile could be used to test the kinetic model under dynamic conditions, but only linear temperature was considered in the dynamic study since kinetic models developed from isothermal conditions could be applied to any time–temperature histories. A linear temperature profile was chosen because it was already developed and tested for temperature control in the circulating water bath in a previous study (Huang, 2003b).

2.5.6. Estimation of bacterial survival during dynamic heating

During dynamic heating, the temperature changed with time, and so did the lethal effect. The total survival had to be evaluated over the entire course of the heating process. The time–temperature history had to be incorporated with the isothermal thermal inactivation kinetics to calculate the survival of bacteria in a thermal process. To calculate the total survival, the differential form of a kinetic model must be used. Denoting Y as the log counts of bacteria ($\log(C)$), the differential form of a thermal inactivation kinetic model (Eqs. 3, 5, 7) can be expressed as

$$\mathrm{d}Y(t) = f(Y,t)\mathrm{d}t. \tag{9}$$

For linear model,

$$\mathrm{d}Y(t) = -\frac{1}{D}\mathrm{d}t; \tag{10}$$

for Weibull-type model,

$$\mathrm{d}Y(t) = -K\alpha t^{\alpha-1}\mathrm{d}t; \tag{11}$$

and for modified Gompertz model,

$$\mathrm{d}Y(t) = \mu(Y_0 - Y)\ln\left(\frac{Y_0 - Y}{Y_0}\right)\mathrm{d}t. \tag{12}$$

Eq. (9) is an initial value ordinary differential equation (ODE) that can be solved either analytically or numerically. For the linear kinetics with a linear heating temperature profile, the differential equation can be solved analytically. For the Weibull-type or the modified Gompertz model, the differential equation must be solved numerically. But all three ordinary differential equations were solved numerically using an ODE procedure in the mathematical package Scilab (version 4.1.1, INDRA, Le Chesnay Cedex, France), which is an open source platform for numerical computation. For the differential form of the modified Gompertz equation, a small value (1.0×10^{-6}) was added to the initial value (Y_0) to allow the initiation of the numerical computation (van Impe et al., 1992; Huang, 2003b). The numerical procedures used in Scilab (version 4.1) were standard and valid methodologies in numerical analysis of ordinary differential equations.

To numerically solve Eq. (9), the temperature-dependent parameters, i.e., the D-value for the linear model, K- and α-values for the Weibull-type model, and μ for the modified Gompertz model, along with time-dependent temperature history, were used in the numerical algorithm. The results of the numerical analysis of each equation under dynamic condition were the changes in the log counts of bacteria as a function of time.

3. Results and discussion

3.1. Isothermal inactivation curves and the shoulder effect

The raw data points shown in Figs. 1 and 2 illustrate typical survival curves of *L. monocytogenes* in ground beef under isothermal heating at 57, 60, 63, and 66 °C. In general, the log counts of bacteria in ground beef gradually decreased with heating time under isothermal conditions. The rate of thermal inactivation under isothermal conditions increased with heating temperature, as shorter time was needed to inactivate the bacteria at higher temperatures. The thermal inactivation curves, however, were not always linear. The survival curves observed at 66 °C were apparently the most linear among all the survival curves. At lower temperatures (57, 60, or 63 °C), the inactivation curves were generally downwardly concaved, which indicates that the rate of inactivation during an isothermal process increased heating time. For heating at 57, 60, or 63 °C, the bacterial concentration did not seem to change significantly at the early stage of the isothermal process. As the heating time exceeded a certain threshold, which varied with temperature, the rate of bacterial inactivation began to accelerate. The typical "shoulder effect", where the log counts of bacteria did not decrease immediately after the bacteria was exposed to heat, was observed in this study.

The "shoulder effect" observed in a heat inactivation study can be caused by the dimension of the samples (Juneja et al., 2006). If a sample used in a heat inactivation study is thick or contained in a large container, the temperature at the geometric center of the sample cannot be increased to the desired heating temperature instantaneously. Time is needed for heat to diffuse into the geometric center of the sample. During this initial time (or come-up time) period, the bacteria located at the geometric center are not significantly affected by the heating temperature. If the sampling time is shorter than the come-up time, the "shoulder effect" will be observed, which is apparently caused by this experimental error. However, the "shoulder effect" observed in this study cannot be attributed to the dimension of the samples or the sampling time since the best effort was undertaken to minimize the thickness of samples and the come-up time (6 s) was excluded from the heating time. At lower temperatures (57 and 60 °C), the sampling times were significantly longer than the come-up time. The "shoulder effect" observed in this study may be attributable to some biological factors where a thermal process must overcome an initial energy barrier before a lethal effect can be observed (Juneja et al., 2006).

3.2. Kinetic analysis of survival curves

Although not all linear, all survival curves can be analyzed with the linear model (Figs. 1 and 2, column A) through linear regression with reasonable accuracy. The R^2 values ranged from 0.870 to 0.986, and were higher with the curves observed at higher temperatures, indicating that the linear model was more

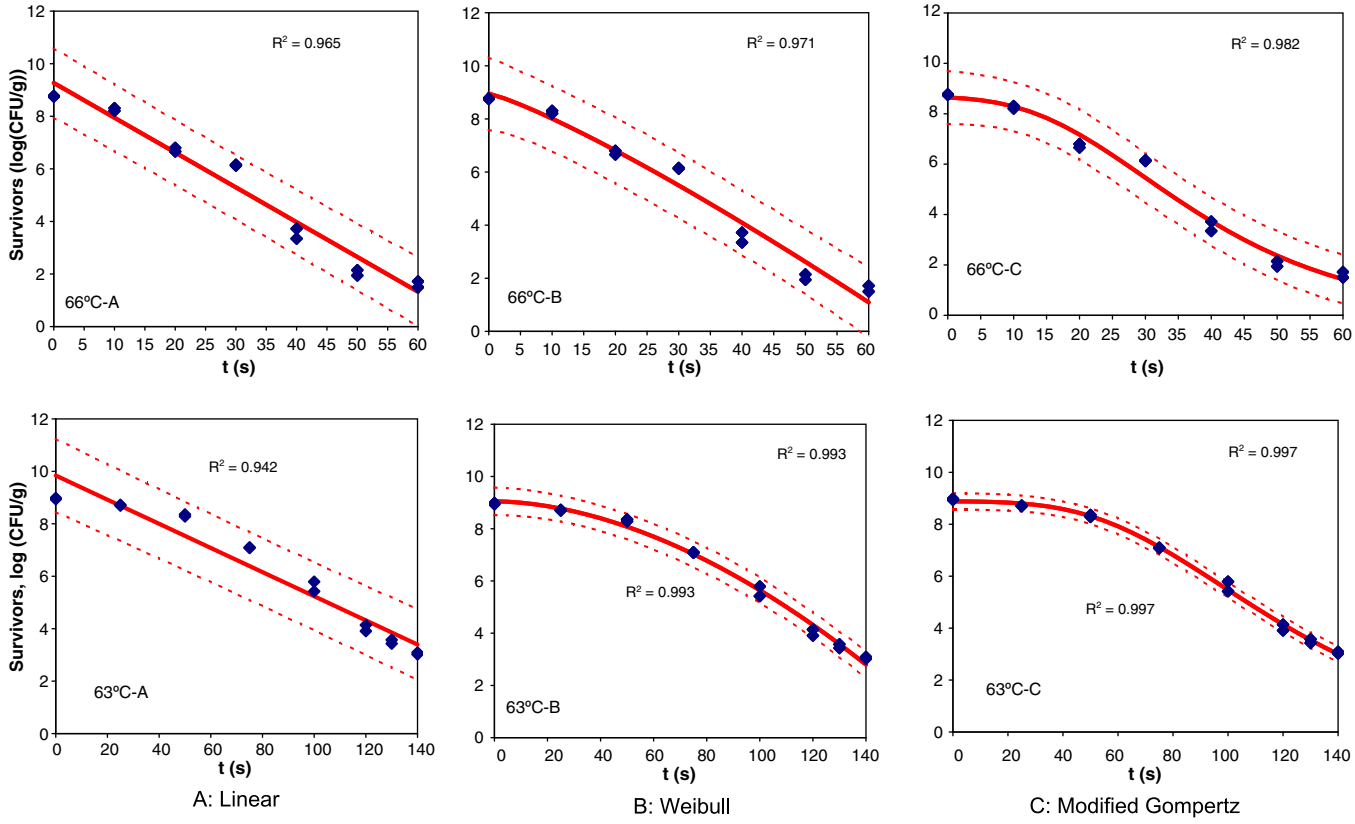

Fig. 1. Representative survival curves of *L. monocytogenes* in ground beef under 66 and 63 °C. Solid diamonds: raw data. The same data set was fitted to linear model (column A), Weibull model (column B), and modified Gompertz model (column C). Dotted curves represent upper and lower prediction limits at 95% confidence intervals.

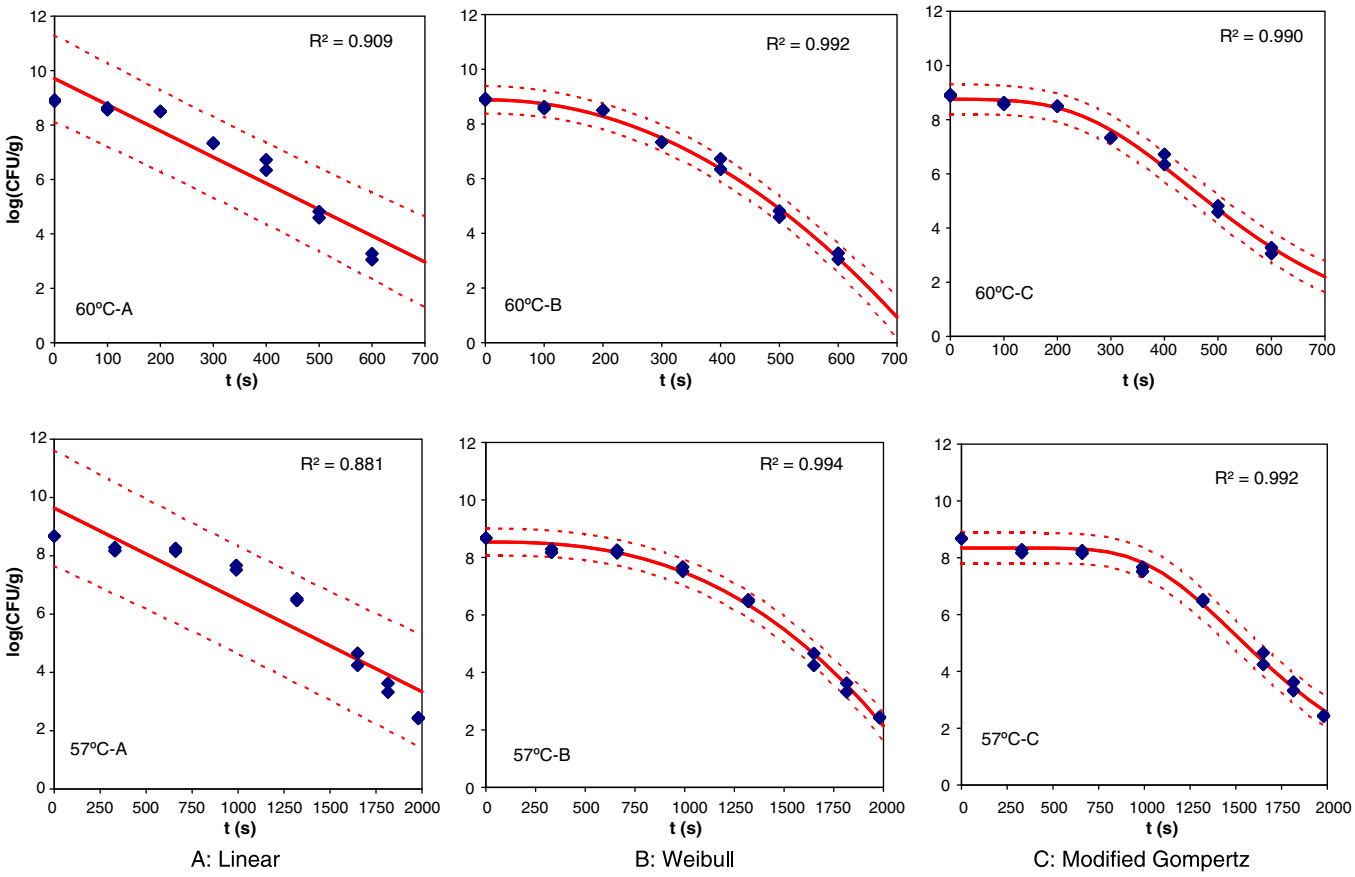

Fig. 2. Representative survival curves of *L. monocytogenes* in ground beef under 60 and 57 °C. Solid diamonds: raw data. The same data set was fitted to linear model (column A), Weibull model (column B), and modified Gompertz model (column C). Dotted curves represent upper and lower prediction limits at 95% confidence intervals.

suitable for describing the inactivation kinetics at higher temperatures. Using the average *D*-values at each temperature, a linear curve ($R^2 = 0.999$) was obtained with the regression between log(*D*) and *T* (Fig. 3). The *z*-value calculated from the regression curve was 5.37 °C. The *z*-values of a 5-strain cocktail in duck muscle, turkey breast, and chicken breast were 5.04, 5.29, and 5.71 °C, respectively (Murphy et al., 2003b). In chicken leg quarter meat, the *z*-value of *L. monocytogenes* was determined as 5.76 °C (Murphy et al., 2003a). For a 6-strain cocktail of *L. monocytogenes* in beef, the *z*-value of was reported as 6.01 °C (Murphy et al., 2004b). Therefore, the *z*-value of the *L. monocytogenes* cocktail obtained in this study is very close to the values reported in the literature.

According to the regression model between log(*D*) and *T*, the *D*-value calculated at 70 °C was 1.27 s. Using 70 °C as a reference temperature, Eq. (4) can be expressed as

$$D = 1.27 \times 10^{-\frac{T-70}{5.37}}. \quad (13)$$

The Weibull-type model was apparently more suitable than the linear model for describing the survival curves observed in this study (Figs. 1 and 2, column B), with pseudo-R^2 values ranged from 0.963 to 0.999. With downward concavity, the power index (α) in Eq. (5) was >1.0. Fig. 4 depicts the effect of temperature on both the rate constant (K) and the power index (α). As illustrated in this figure, the rate constant (K) of the Weibull-type increases exponentially with the heating temperature ($R^2 = 0.996$):

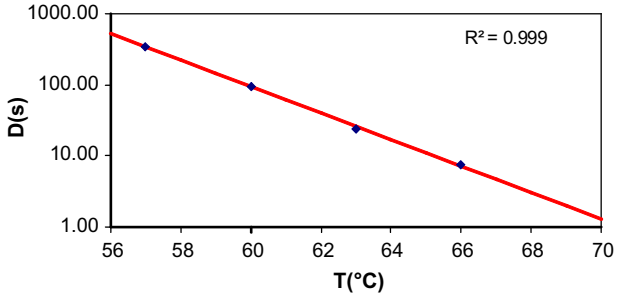

Fig. 3. Effect of temperature on *D*-values – the linear model. ♦: Average *D*-values at each temperature and —: the regression curve between log(*D*) and *T*.

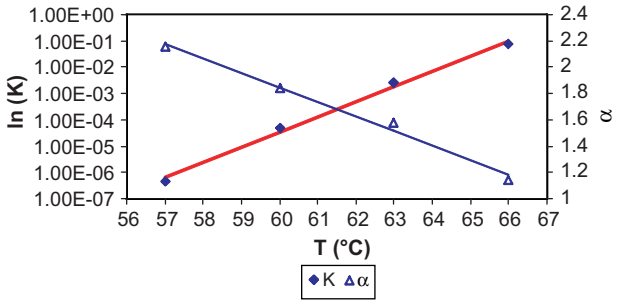

Fig. 4. Effect of temperature on the average coefficients (k and α) of the Weibull-type model. ♦: Average *K*-values at each temperature and Δ: average α-values at each temperature.

$$\ln(K) = -90.33 + 1.33T. \quad (14)$$

The power index (α) decreases linearly with the heating temperature (Eq. (15), R^2 = 0.990), indicating that the survival curves lost the downward concavity gradually as the temperature increases. At 57 °C, the average power index was about 2.15. But at 66 °C, the α-value was only 1.2, which indicates that the survival curve at this temperature was very close to linear. Since it was not physically possible to sample data at temperatures above 66 °C, the trend of α at temperatures higher than 66 °C is not clear.

$$\alpha = 8.46 - 0.110T \quad (15)$$

The survival curves also can be fitted to the modified Gompertz model (Figs. 1 and 2, column C). The pseudo-R^2 values of the modified Gompertz model ranged from 0.971 to 0.999. According to these figures, the "shoulder effect" is better described by the modified Gompertz model, where the curve would exhibit a "plateau" at the early stage of the heating process and after which the kill rate increased significantly in an almost a linear manner (Figs. 1 and 2, column C). Both μ and M were affected by temperature (Fig. 5), with μ increased exponentially with temperature (Eq. (16), R^2 = 1.0), while M decreased exponentially with temperature (Eq. (17), R^2 = 0.999).

$$\ln(\mu) = -29.0 + 0.095T \quad (16)$$

$$\ln(M) = 32.9 - 0.449T \quad (17)$$

The mean values of RMSE were 0.48, 0.20, and 0.19 log(CFU/g), respectively, for the linear, Weibull-type, and modified Gompertz models. RMSE is the standard error of the estimation by the models, and is an estimate of the standard deviation of the bacterial inactivation calculated by the models. According to the ANOVA analysis, the mean of RMSE obtained from the linear model was significantly higher than those from the modified Gompertz and Weibull-type models ($p < 0.05$). However, there was no significant difference in the means of RMSE between the modified Gompertz and Weibull-type models. The ANOVA analysis suggests that the Weibull-type and modified Gompertz models are equally suitable for describing the process of thermal inactivation of *L. monocytogenes* in ground beef and have smaller standard errors than the linear model under isothermal conditions.

3.3. Validation of kinetic models under dynamic condition

The kinetic models developed in this study were validated with a defined linear temperature profile (Fig. 6). The temperature of the water was automatically controlled to increase linearly from 30 °C to a final temperature around 65 °C. The average heating rate was 1.72 °C/min. With a defined heating profile, the differential form of the kinetic model (Eq. (9)) was directly solved using a numerical method (Fig. 6). To check the accuracy of the numerical method, the analytical solution to the linear kinetic model was obtained (Eq. (18)). The results of the numerical analysis to the linear kinetic model were almost identical to the analytical solution, which validated the accuracy of the numerical techniques used in this study (Fig. 7).

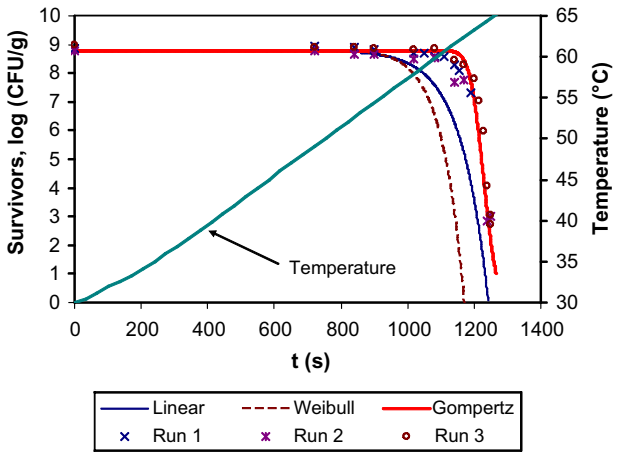

Fig. 6. Survival of *L. monocytogenes* in ground beef during dynamic heating.

$$Y = Y_0 - \frac{60 \times 5.37}{1.27 \times 1.72 \times \ln(10)} \left(10^{\frac{1.72t}{60\times5.37} - \frac{40}{5.37}} - 10^{-\frac{40}{5.37}}\right) \quad (18)$$

Eq. (18) is the analytical solution to Eq. (10) under a linear heating temperature profile with a heating rate 1.72 °C/min. Y and Y_0 are the logarithm of the real-time and initial counts of bacteria. Judging from Fig. 6, it is evident that the modified Gompertz model was the most accurate kinetic model for estimating the survival of *L. monocytogenes* in ground beef under dynamic conditions, while both of the linear and Weibull-type models grossly underestimated the survival of the bacteria during heating. The RMSE calculated from the modified Gompertz model was 0.71 log(CFU/g).

It is relatively easy to explain the reason why the linear model would underestimate the survival of bacteria during dynamic heating when the downwardly concaved isothermal survival curves were observed during isothermal studies. In a linear model (Eq. (3)), $1/D$ represents the average rate of bacterial inactivation. However, for a downwardly concaved survival curve, the Y-intercept obtained from linear regression would always overestimate the initial concentrations (Figs. 1 and 2, column A). The regression line is actually an averaged thermal inactivation curve. When the

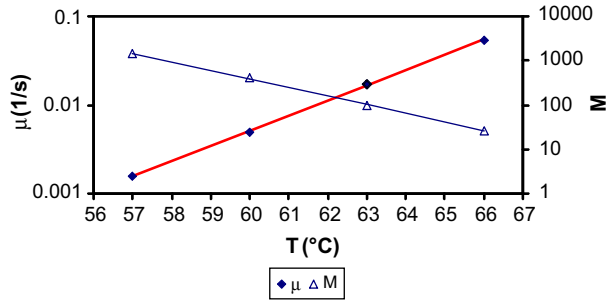

Fig. 5. Effect of temperature on the average coefficients (μ and M) of the modified Gompertz model. ♦: Average μ-values at each temperature and Δ: average M-values at each temperature.

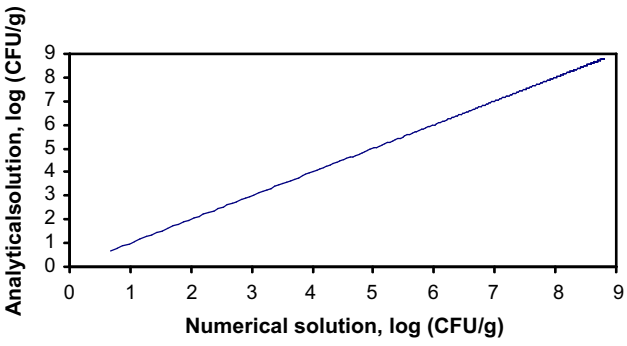

Fig. 7. Comparison between the results of analytical and numerical solutions to the linear model (Eq. (10)) during linear heating.

actual initial concentration and the *D*-value are used to calculate the survival of bacteria even under an isothermal condition, the calculated survival curve would have to start from the actual initial concentration, and is parallel to, but below the averaged thermal inactivation curve. As a result, the calculated thermal inactivation curve would underestimate the survival of bacteria even under isothermal conditions. When the *D*- and *z*-values obtained from the isothermal conditions are used to estimate the survival of bacteria under dynamic conditions, the errors may accumulate and propagate, resulting in an overall underestimation of the survival of *L. monocytogenes* in ground beef (Fig. 6).

The Weibull-type model is very accurate when used to describe the individual survival curves. The pseudo-R^2 values of the Weibull-type models were very close to those of the modified Gompertz models. However, the Weibull-type models were the least accurate among the three models used to estimate the survival of *L. monocytogenes* in ground beef under dynamic conditions. The use of the Weibull-type model would grossly overestimate the extent of bacterial inactivation during dynamic heating. The inaccuracy of the Weibull-type model for estimating the bacterial inactivation in a dynamic process may be attributable to the model itself. For a downwardly concaved curve, the power index α is >1.0. In the differential form (Eq. (11)), the rate of bacterial inactivation is directly proportional to $t^{\alpha-1}$. Since $\alpha - 1$ is >0, $t^{\alpha-1}$ is always >1 when $t > 1$ s, making dY/dt sensitive to t, particularly when t is very large. In combination with K, which increased exponentially with t in a linear heating temperature profile, dY/dt would accelerate as the heating process progresses, leading to an overall overestimation of bacterial inactivation in a dynamic process. The most significant drawback of the Weibull-type model, at least observed in this study, may be that the index for curve shapes, α, is dependent upon temperature. Since both K and α are highly dependent upon the heating temperature, and the primary (Weibull-type) model is not perfect even under isothermal conditions, the computational errors may be amplified by the α-values. The computational errors are not caused by the numerical method, but by the primary model itself.

4. Conclusions

This study directly compared three mathematical (linear, Weibull-type, and modified Gompertz) models used to describe the inactivation of *L. monocytogenes* in ground beef under both isothermal and dynamic conditions. Under isothermal conditions, the survival curves of *L. monocytogenes* in ground beef did not strictly follow the first-order kinetic model, but exhibited downward concavity. Under isothermal conditions, the Weibull-type and modified Gompertz models are both more suitable than the linear model for describing the survival curves. However, under a dynamic condition where the temperature was increased linearly from 30 to 65 °C, the linear and Weibull-type models both grossly underestimated the survival of the bacteria, and were not suitable for estimating the bacterial survival in a dynamic process. The modified Gompertz model was more accurate than the linear and Weibull-type models in estimating the survival of *L. monocytogenes* in ground beef under dynamic conditions. The standard error of the modified Gompertz model when used to estimate the survival of bacteria in the dynamic thermal process was only 0.71 log(CFU/g), making it the only one among three models capable of quantifying the survival of *L. monocytogenes* in ground beef in a dynamic process.

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